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# **Crystal structure of human Leukotriene C4 synthase – an integral membrane protein in the synthesis of inflammatory mediators**

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# Integral membrane protein (IMP) structural biology

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## Urgent need for IMP structures

- Understanding of many membrane protein processes still rudimentary due to lack of structural information
- Integral membrane proteins of outstanding importance as drug targets.

## Current state

- Less than 120 unique IMPs structures determined, majority from prokaryotes
- Current rate, 5-10 novel alpha-IMP structures per year
- Only ~ 5 eukaryotic IMPs structures from recombinant protein
- Jan 2007: only one human IMP structure, an aquaporin electron diffraction structure at 3.7 Å

## Membran protein technologies and structures:

Gustafsson foundation (2002-2005)

SPINE (EU) (2001-2005)

Karolinska Institute (2005=>)

E-MEP (EU) (2004=>)

ECOISANOX (EU) (2005=>)

=> 2-5.5 people

- Technologies for parallel IMP production
- 2 novel membrane protein structures in last two years

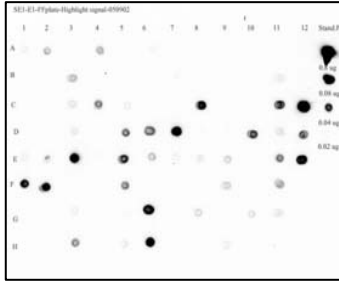
Dept. of Medical Biochemistry and Biophysics



Divisions of; Biophysics, Structural biology and Structural Genomics Consortium (SGC); (PIs: Pär Nordlund, Said Eshaghi, Gunter Schneider, Ylva Lindqvist, Doreen Dobritzsch and Johan Weigelt)

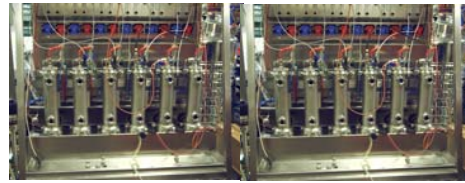
# The “Stockholm platform”: Enabling Parallel Technologies for Protein Production for Structural Studies

## (1) Expression screening



• Parallel and semi-automated expression screening, solubilization and affinity purification

## (2) Parallel Scale-up in the 1 Litre multi-fermentor *Greta*

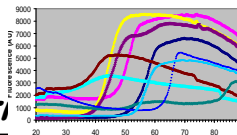


## (3) Purification

Streamlined *ÄKTA*-based multi-step purification and scouting



(4) Biophysical checkpoint  
*CD*, *MS*,  
*Thermoflour* for construct and additi



Parallel processes applied on:

**Soluble proteins:**

-E.coli, Herpes and Human

**Integral membrane proteins:**

- E.coli, T. Maritima and Human

5) Directed evolution of “protein expression and stability”.

• e.g. GFP or FIDO based screening of expressibility, stability and solubility



*Crystallization*  
automated Screening

# Tentative success rates for expression and purification of IMPs (small-scale)

**E.Coli - single expression condition - His-tagged proteins**

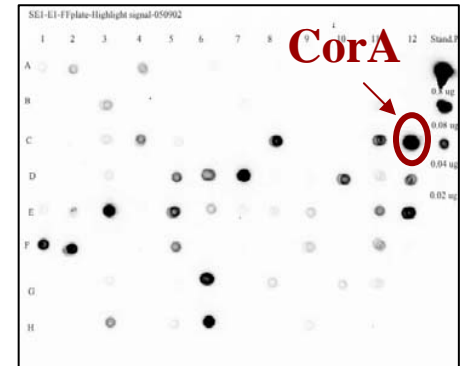
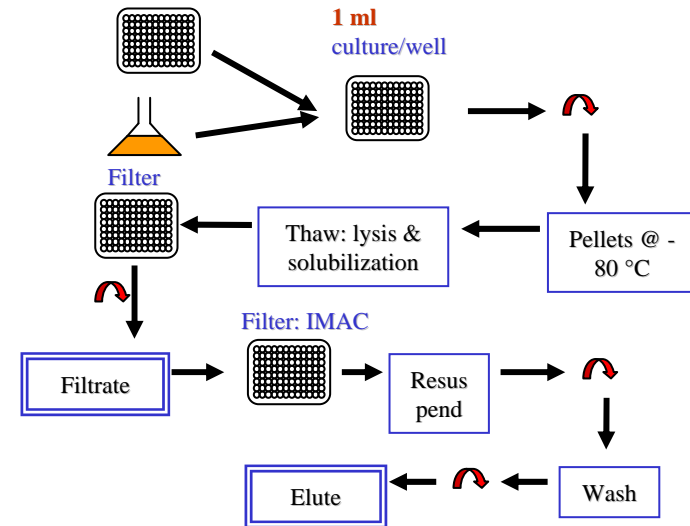
**Homologous expression:**

*E.coli* IMPs from 30+ families (#48) >50%

**Heterologous expression (Obs, in different vectors)**

- *Thermotoga maritima* IMPs (#168) >20%

- **Human** IMPs (#485) >17%



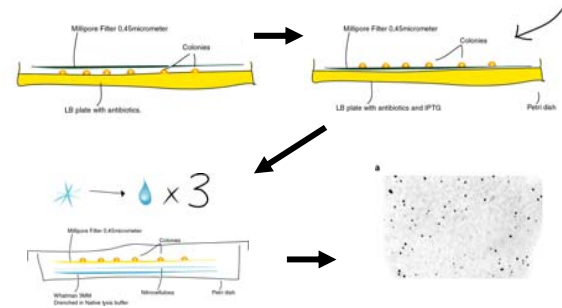
(Eshaghi Prot Science, 2005)

⇒ Typical >2/3 of these proteins can be scaled-up for crystallization trial

Current focus in group on ~15 structural families

# Directed evolution - Selection of mutations which improve levels of detergent purifiable IMPs

508 | VOL.2 NO.7 | JULY 2005 | NATURE METHODS

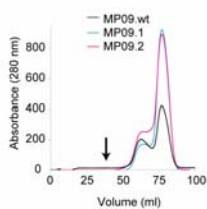


**E.coli 1**



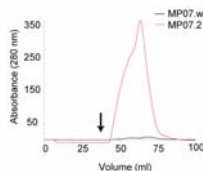
No positive colonies

**Human 4**



**3.5-fold more IMP**

**E.coli 7**

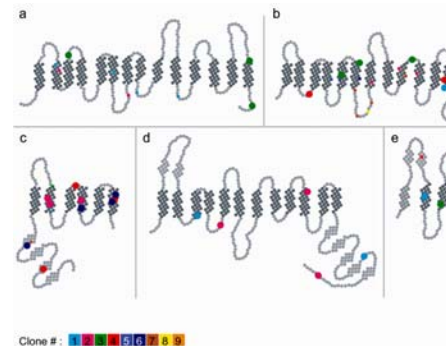


**40-fold more IMP**

- Accumulated data on mutations improving expression/detergent solubilisation will eventually give knowledge base of useful mutational strategies

⇐ Detergent adapted CoFi-blot of libraries of random mutated IMP ORFs

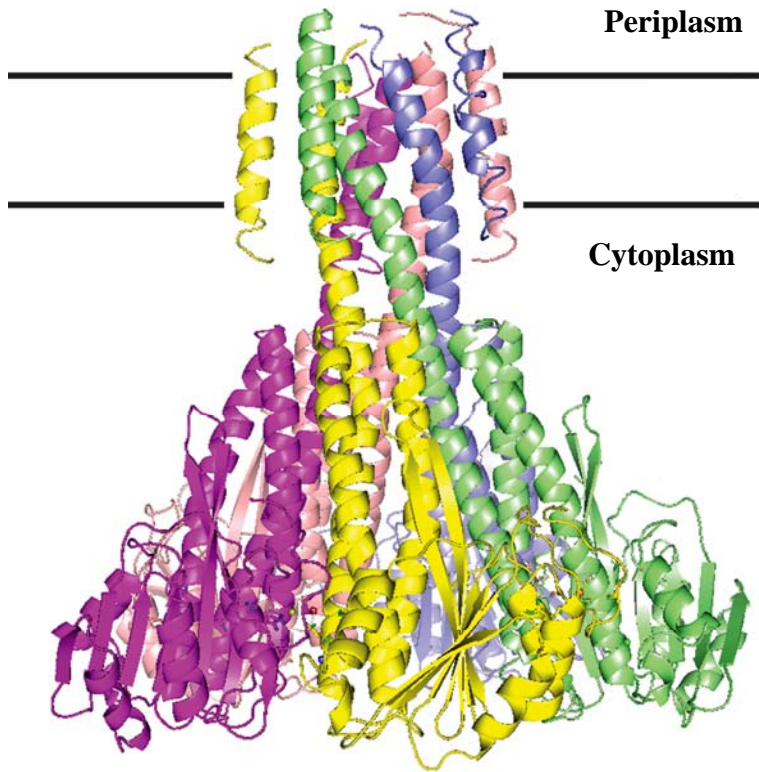
⇐ Scale-up purification of selected mutant vs. non-mutated IMP.



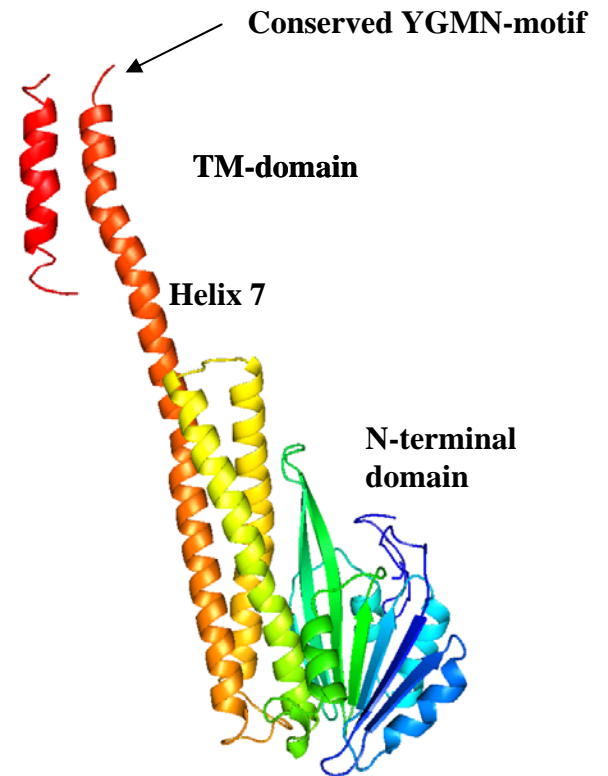
# Time-Line for our two structures

- **CorA**
- September 2005
  - 168 TM clones screened
  - First crystals of CorA diffract to  $\sim 10$  Å
- October 2005:
  - Optimization of crystals to under 4-5 Å
- December 2005:
  - Complete data set at 2.9 Å
  - A low resolution structure at 3.9 Å was determined by SGC Toronto, now published in Nature.
  - Structure solved at 2.9 Å by MR using the Toronto 3.9 Å structure
- **LTC<sub>4</sub>S**
- September 2006
  - Protein expressed in Pichia
  - Purification including His-tag, ligand affinity step & GF
  - Detergent optimization using small scale-platform
- October 2006
  - Crystallize in several conditions
  - Full data set of apo-protein at 2.0 Å
- November 2006
  - Structure solved with heavy atoms (2.0 Å / 2.2 Å)

# CorA divalent Metal transporter



**Pentamer**

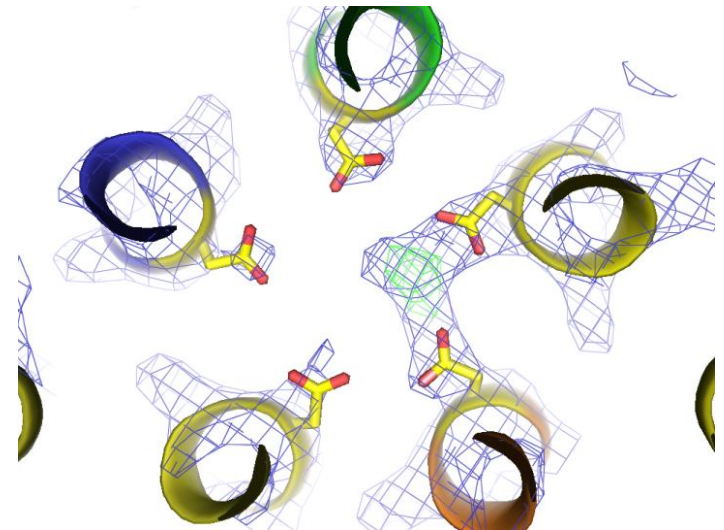
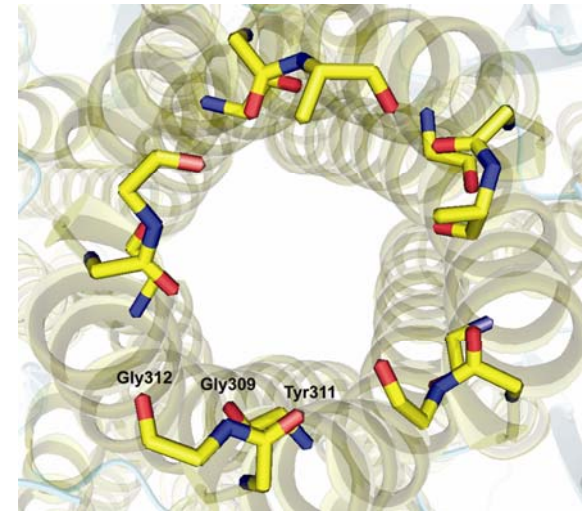
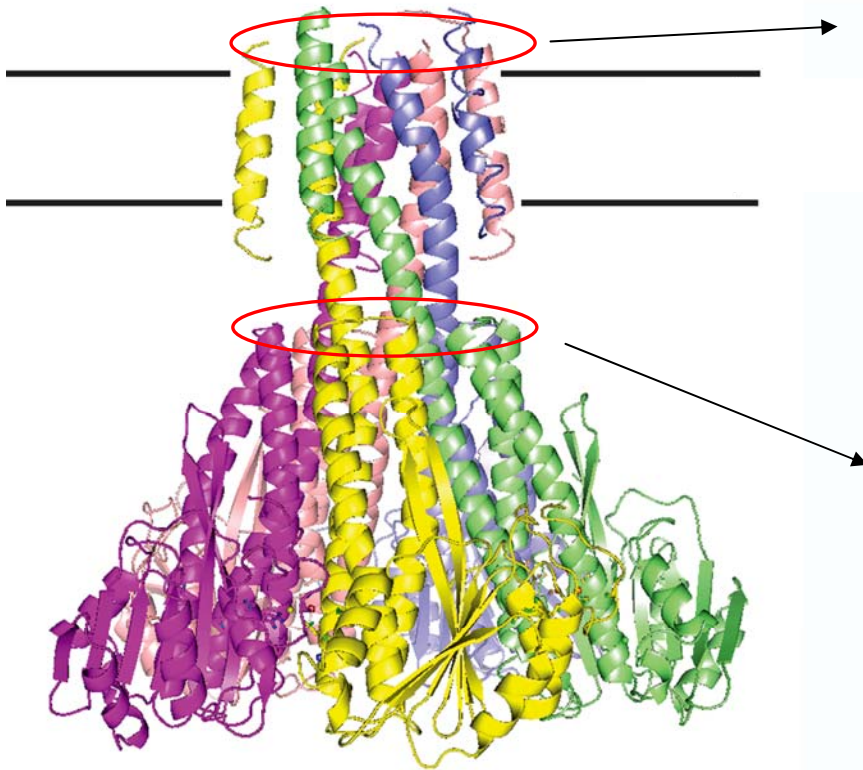


**Monomer**

Eshaghi et al, Science July 2006



# CorA $M^{2+}$ transporter at 2.9Å



Polar environment may assist in dehydration/rehydration of divalent cations

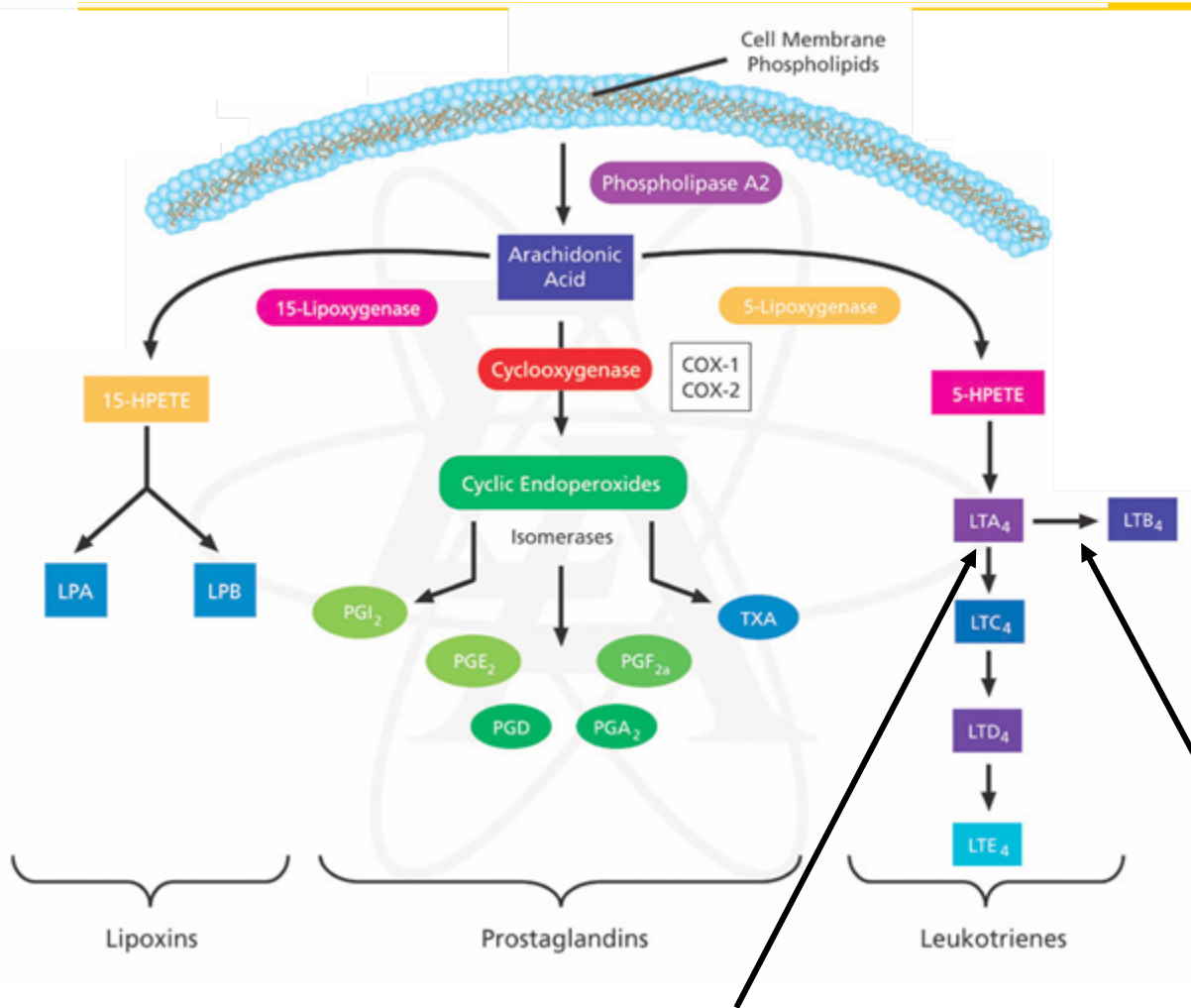
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**Nature, Aug 2, 2007**

# **Structural basis for synthesis of inflammatory mediators by human leukotriene C<sub>4</sub> synthase**

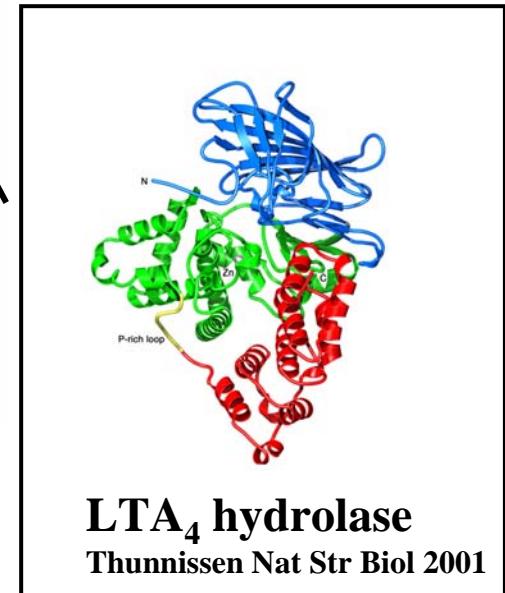
Daniel Martinez Molina<sup>1,4</sup>, Anders Wetterholm<sup>2</sup>, Andreas Kohl<sup>1</sup>, Andrew A. McCarthy<sup>5</sup>, Damian Niegowski<sup>1,4</sup>, Eva Ohlson<sup>2</sup>, Tove Hammarberg<sup>2</sup>, Said Eshaghi<sup>1</sup>, Jesper Z. Haeggström<sup>2</sup> & Pär Nordlund<sup>1,3</sup>

# Arachedonic Acid signaling

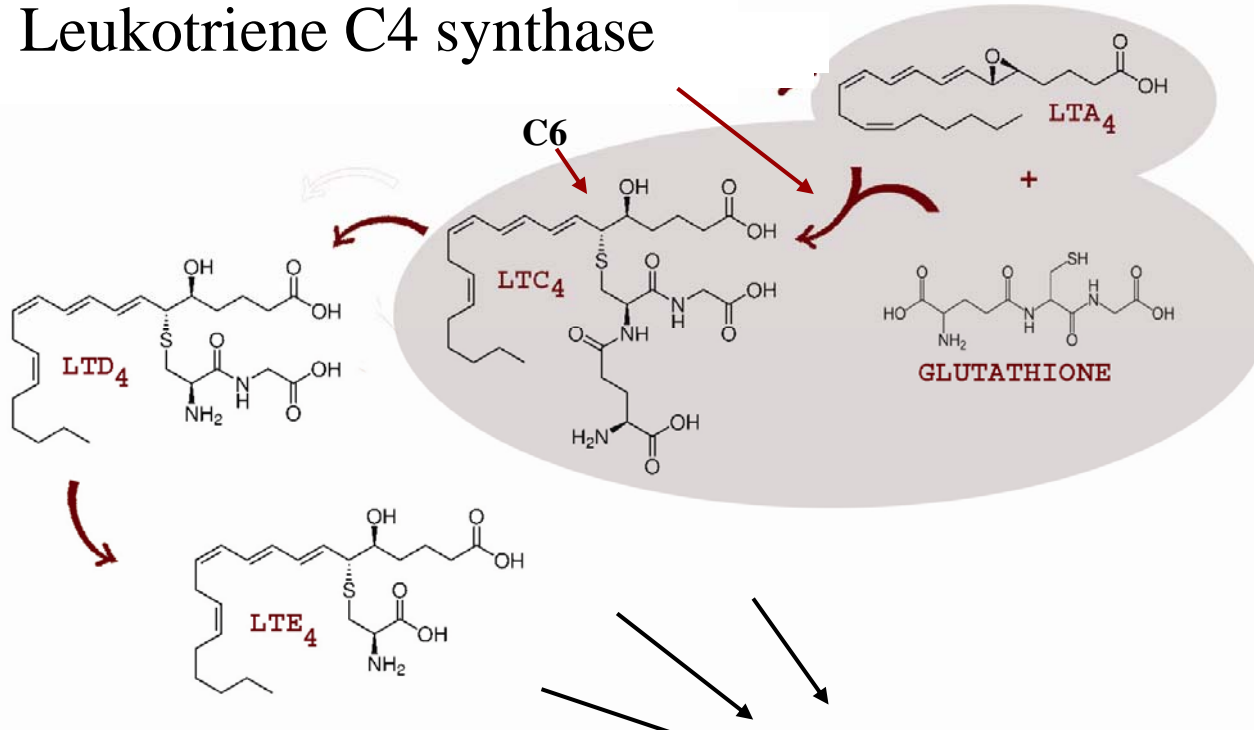


Pathways produce inflammatory and regulatory mediators involved in a wide range of physiological and pathophysiological responses.

**Leukotriene C4 synthase**



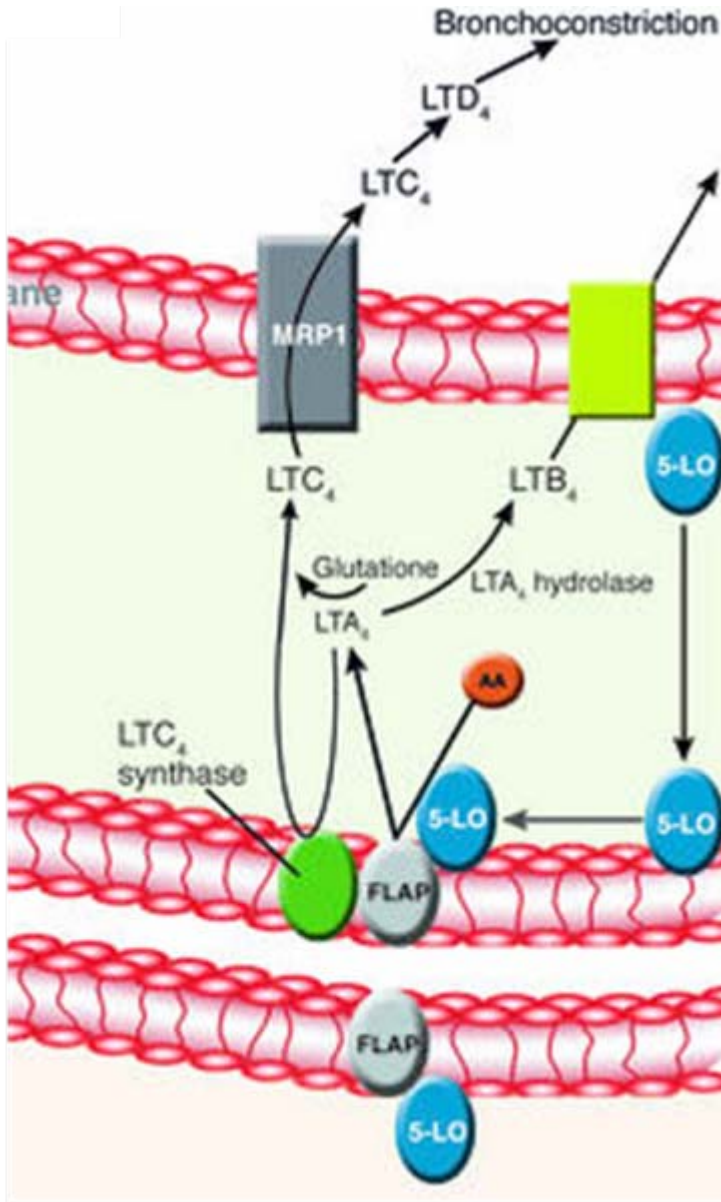
## Leukotriene C4 synthase



Inflammation and Asthma responses mediated by e.g. cysLT1 and cysLT2 receptors.

**Receptor antagonists promising therapeutics for asthma therapies (e.g. montelukast)**

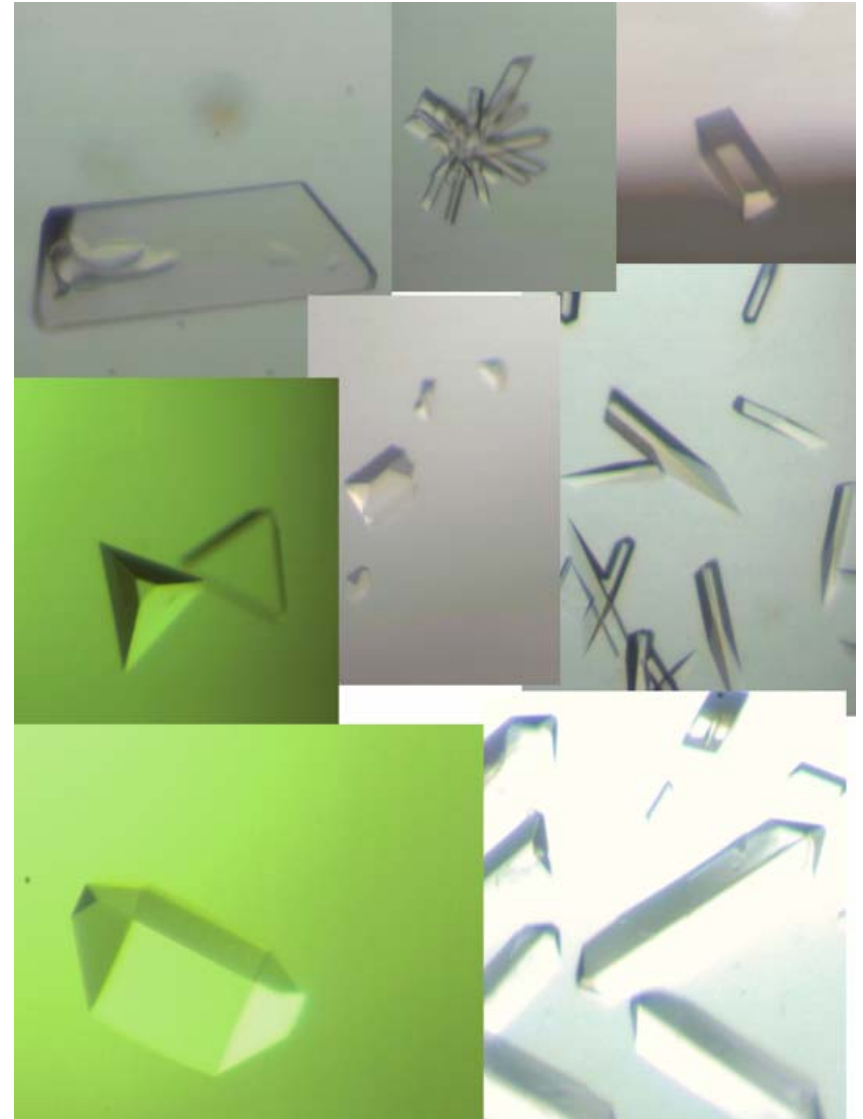
# Leukotriene C4 synthase



NM

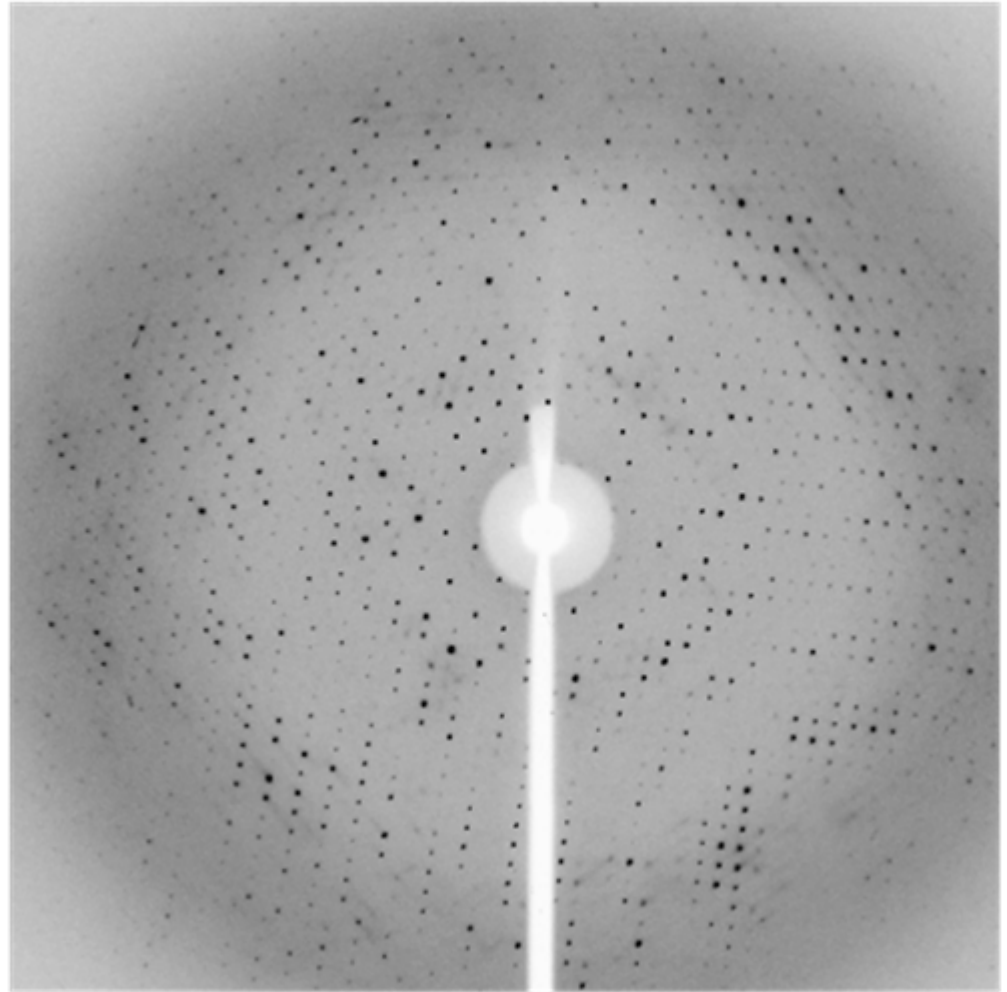
- Member of the MAPEG family (Membrane Associated Proteins in Eicosanoid and Glutathione metabolism)
- Located in the outer nuclear membrane and peripheral endoplasmic reticulum
- Leukotriene C4 synthesis is potential spatially coordinated

- Rat and Human LTC<sub>4</sub>S expressed in *Pichia pastoris*
- Purified using His and GSH affinity columns, plus GF
- Crystals from rat construct diffracted to 6 Å at best
- Large difference in diffraction between detergents - DDM best
- Crystals from new human construct optimized (<2.0 Å)

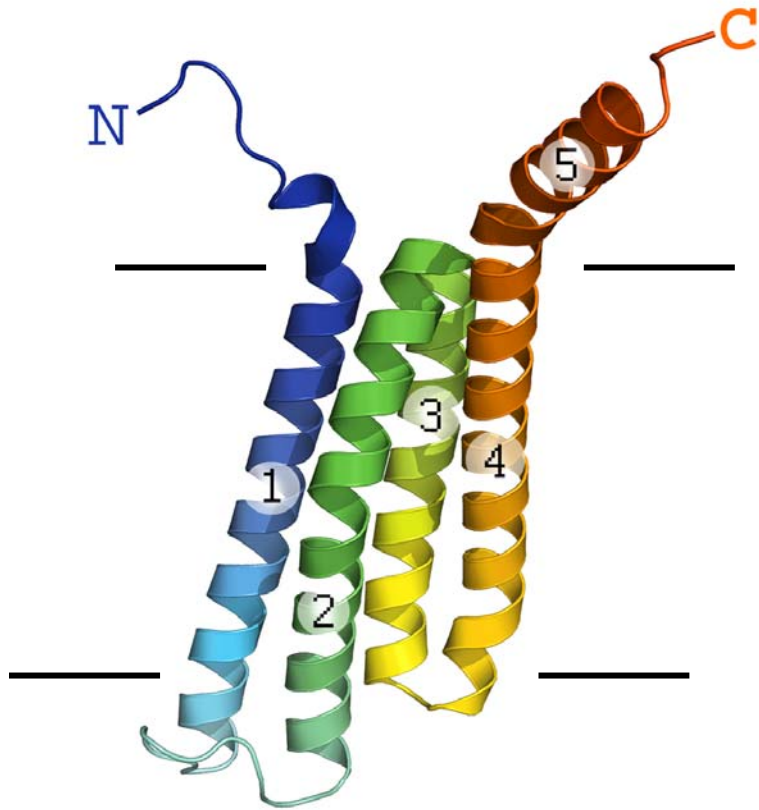


# Leukotriene C4 synthase

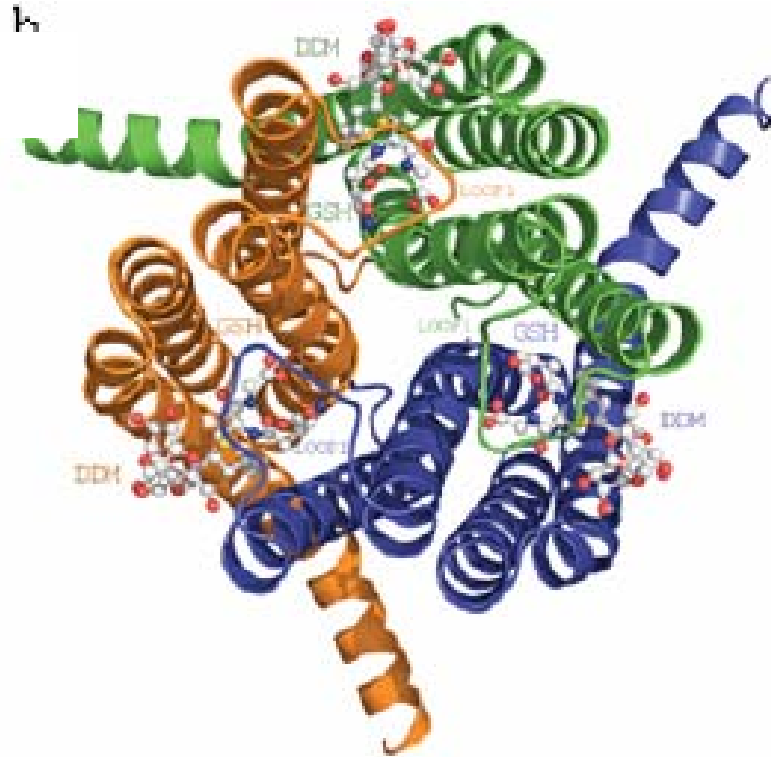
- Structure determined using MAD on Pt derivative
- Glutathione (GSH) soaked crystals diffract to 2.2 Å
- Space group F23 (196)
- > 1000 crystals screened for native, HA derivatives and GSH complexes; at ESRF, BESSY, SLS, and MaxLab



# Structure of LTC<sub>4</sub>-synthase



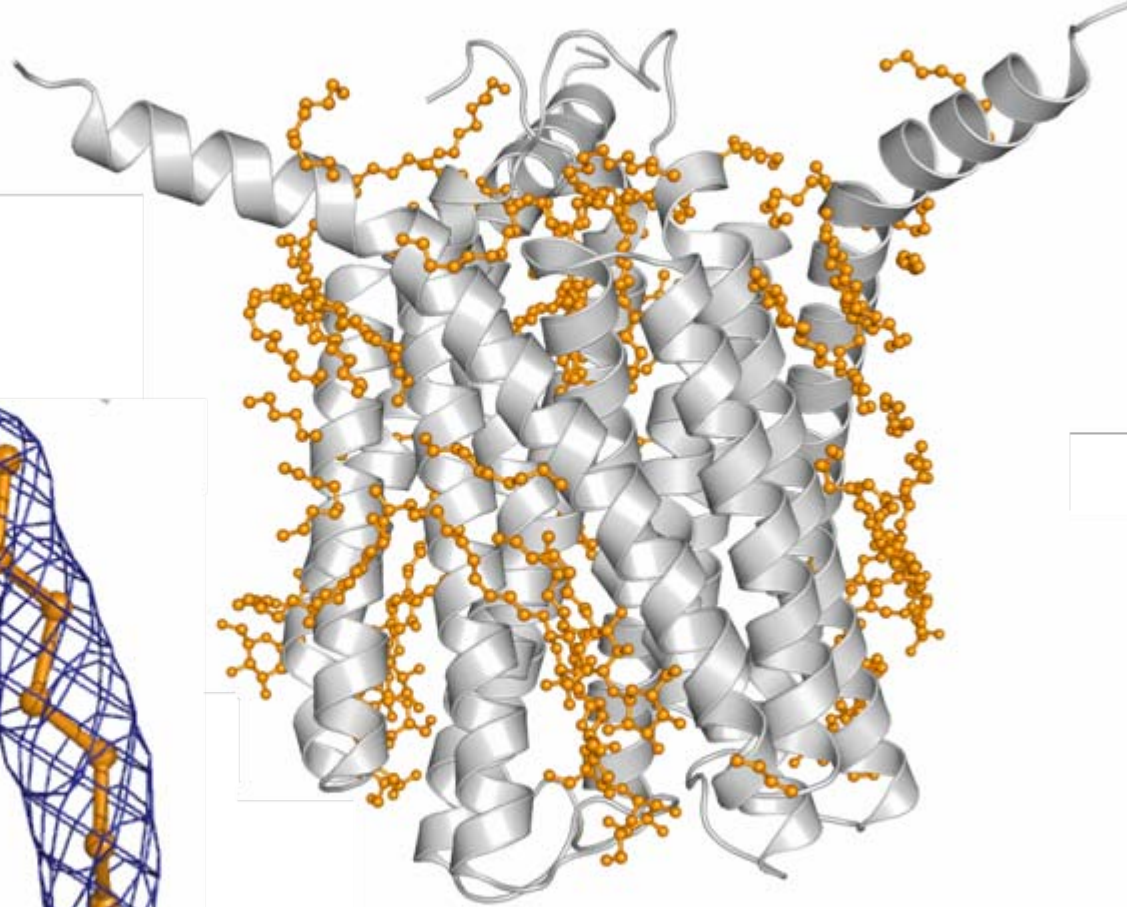
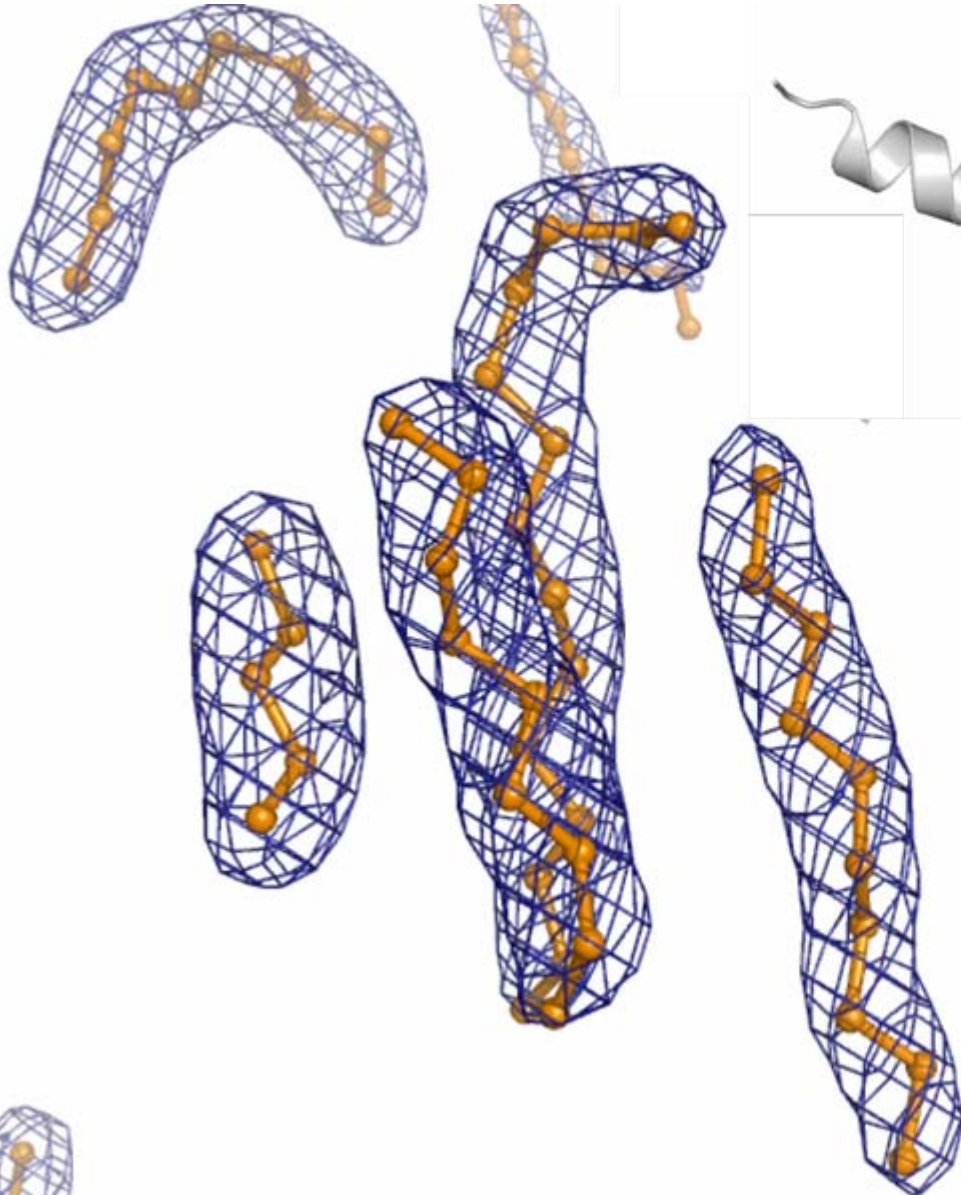
**Monomer (150aa)**



**Trimer**

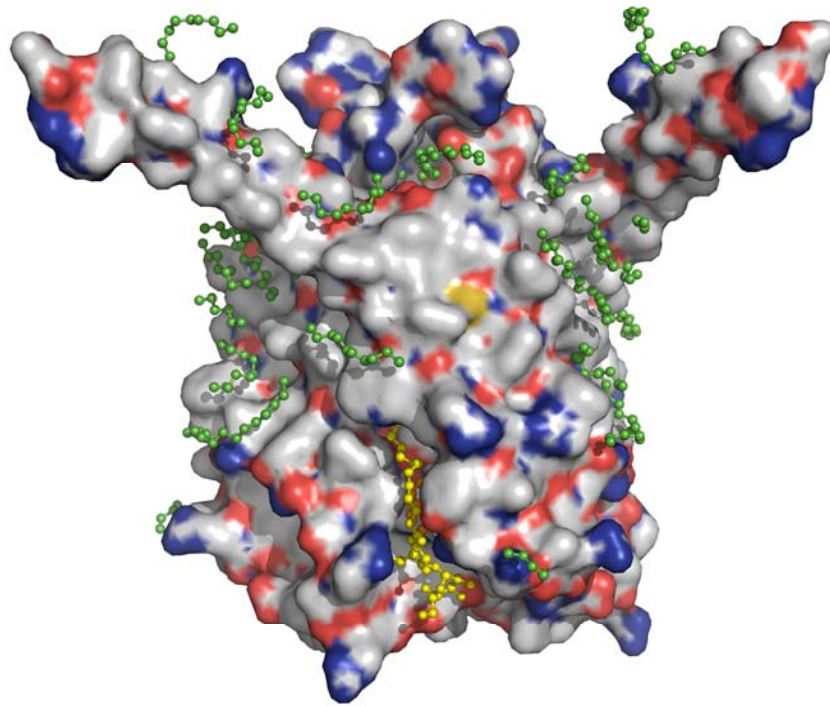


# Aliphatic chains of detergents/lipids



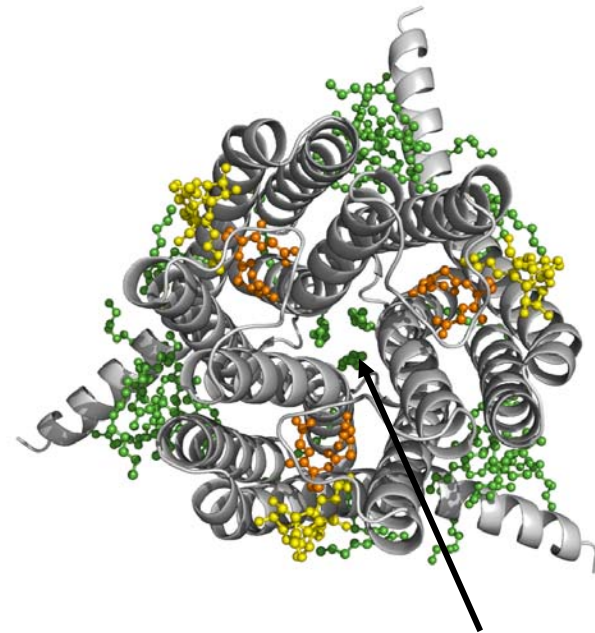
Stretches of density modelled as carbon chains if 4-18C

# Detergent-lipid binding



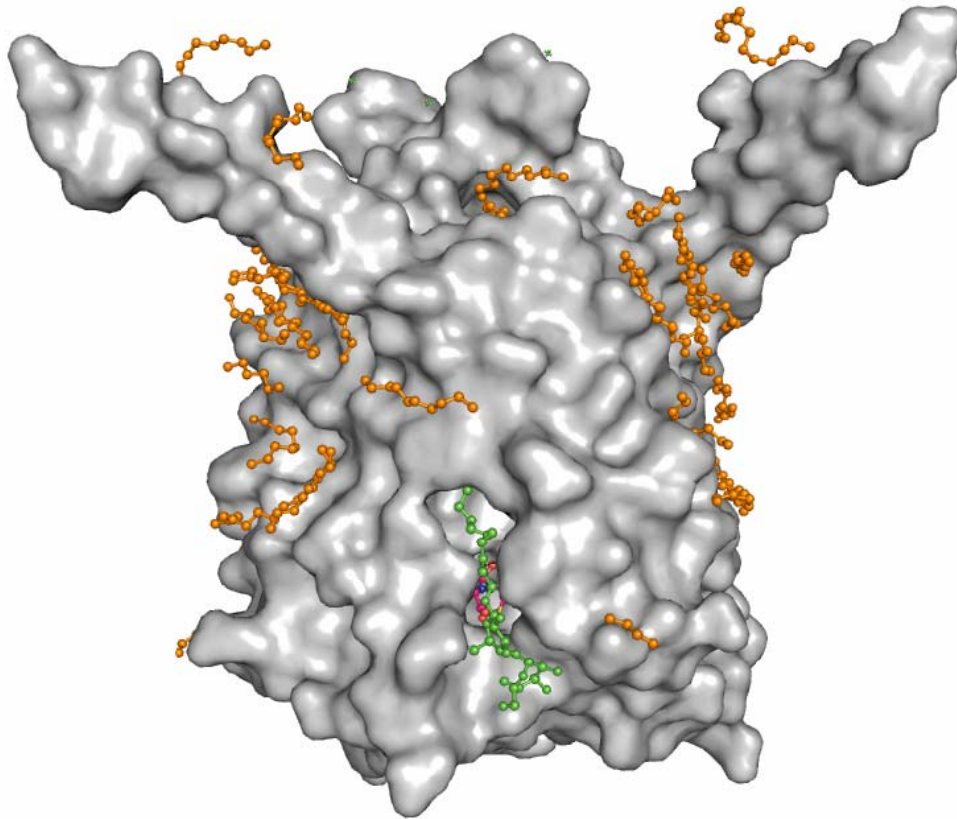
**Identity of most aliphatic chains unknown**

**Helix 5 might be interfacial with membrane**



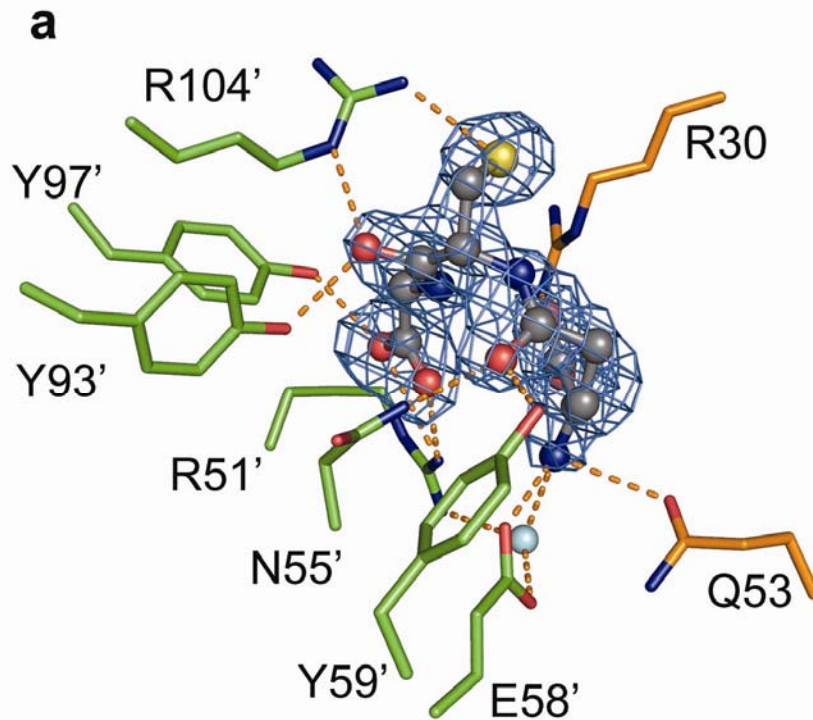
**Lipids found in central cavity**

# The active site

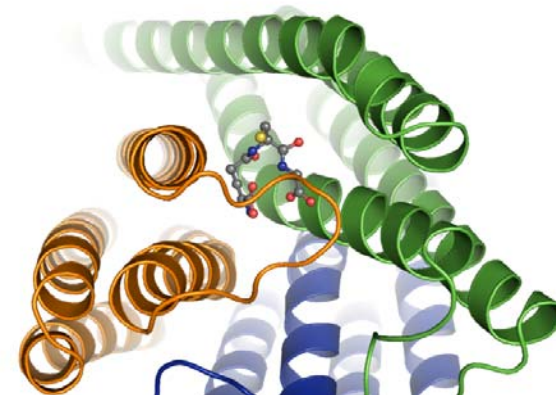


- **AS located between two subunits**
- **3 active sites/trimer**
- **Cytosolic “entrance”**
- **Glutathione cavity is covered by DDM molecule**

# Glutathione binding site

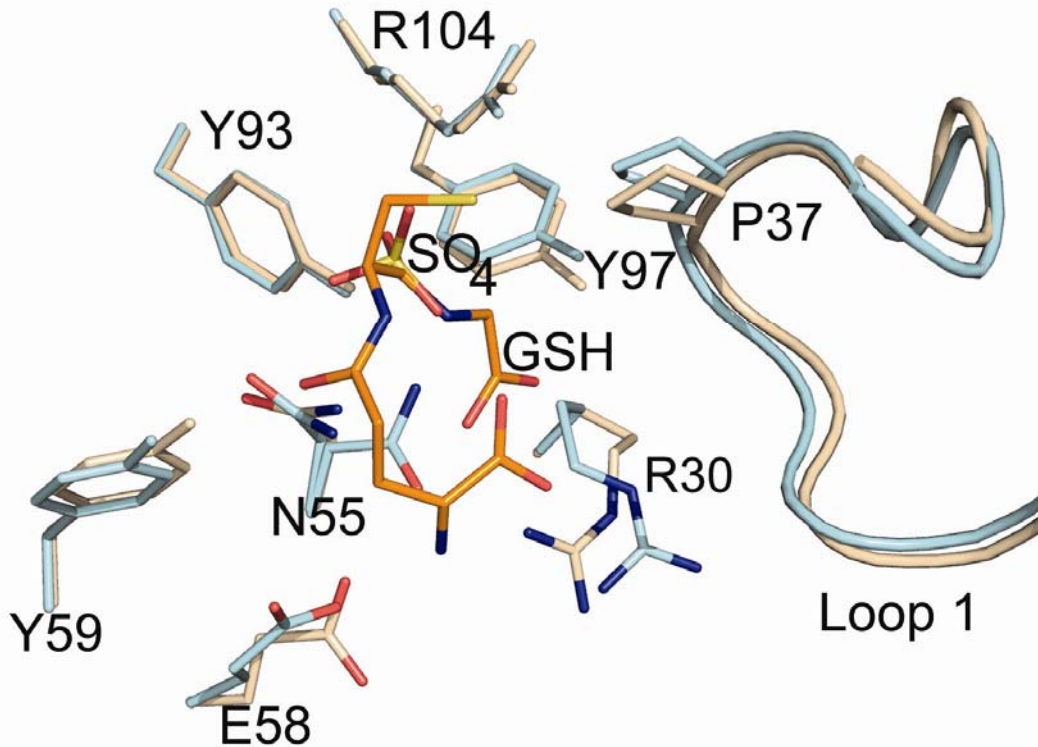


- GSH binds in horseshoe shaped conformation
- Most GSH binding residues conserved
- Arg 104 well positioned to activate SH of GSH
- GSH might be a thiolate in the crystal



# GSH v.s. “Apo” structures

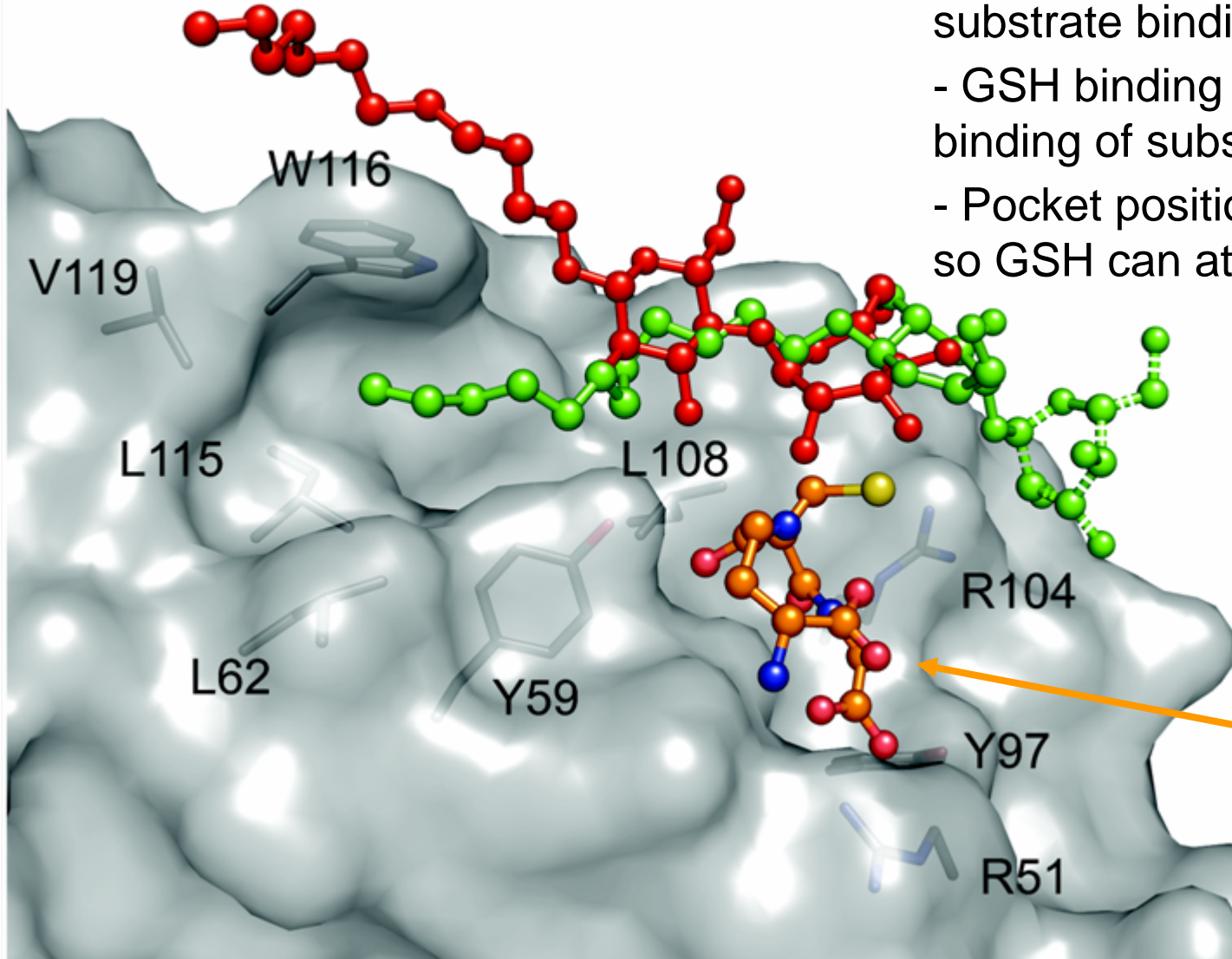
**b**



- $\text{SO}_4$  bound in “apo-LTC4S structure”
- Polar residues change conformation upon GSH binding.
- Only small conformational changes of aromatics
- Loop 1 restrained by crystal contact

# Substrate recognition The active site - Summary

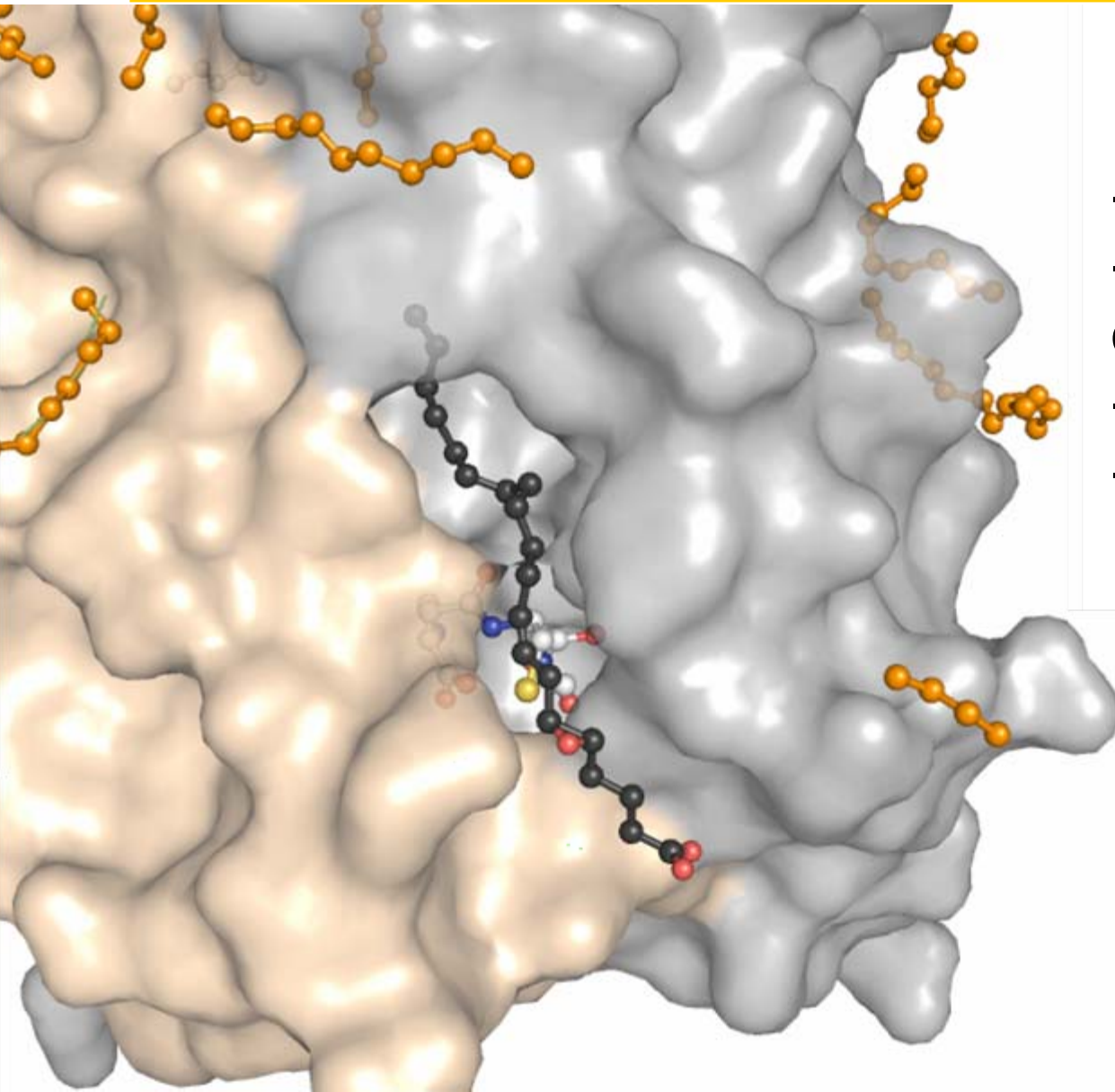
- DDM molecule appears to mimic substrate binding
- GSH binding induces productive binding of substrate LTA4
- Pocket positions substrate (/DDM) so GSH can attack C6



**DDM in:**  
**GSH compl**  
**Apo struct.**

**GSH**

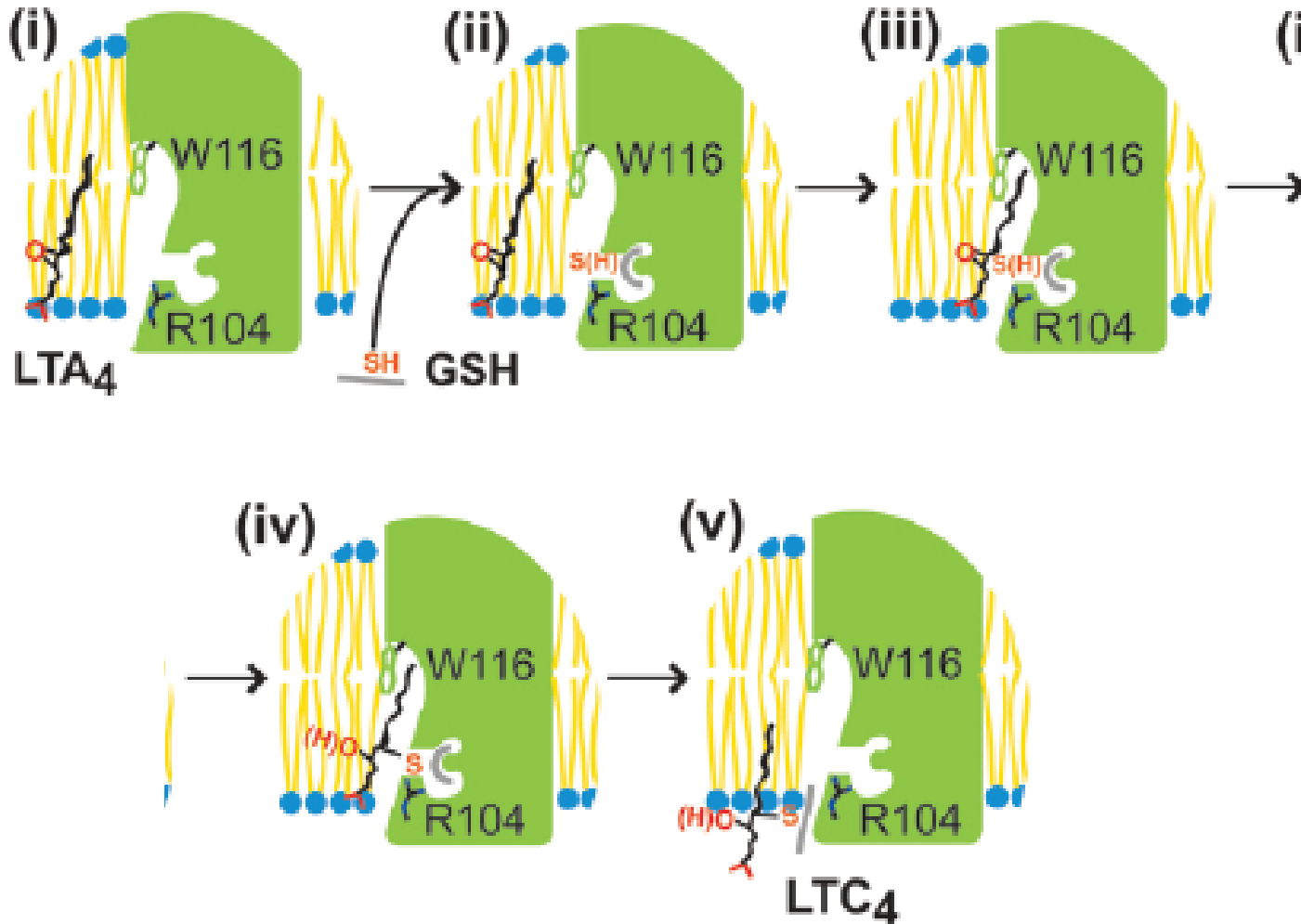
# Specificity for LTA4



- The head group
- Length of aliphatic chain
- The kinks
- A “molecular ruler”

Less conservation among residues lining the LTA4 binding crevice than those coordinating GSH in MAPEG family

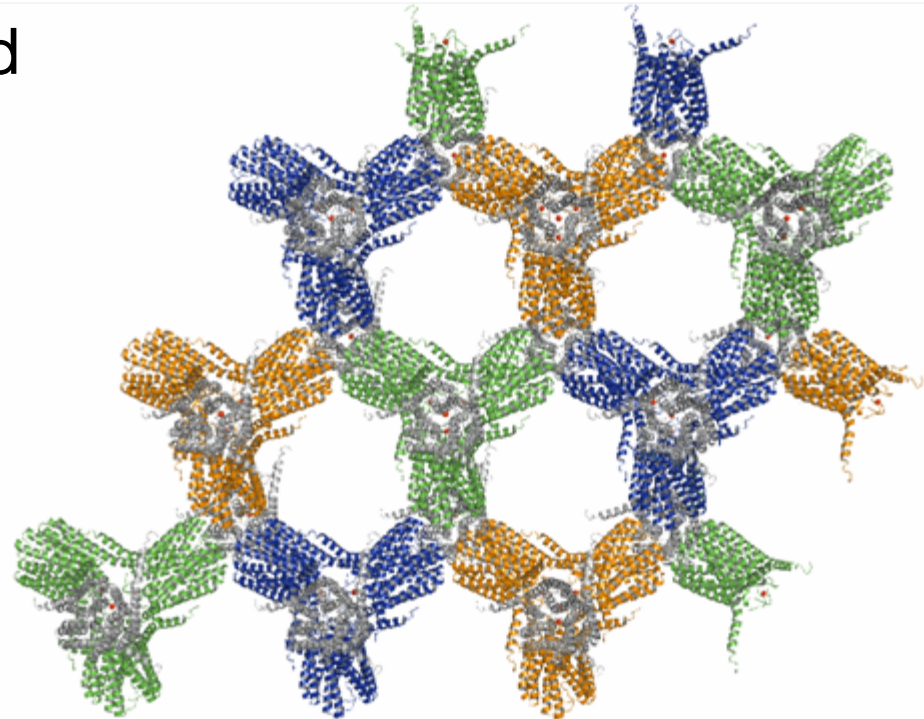
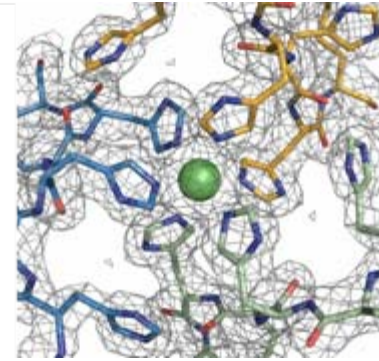
# Reaction scenario of LTC<sub>4</sub> synthase



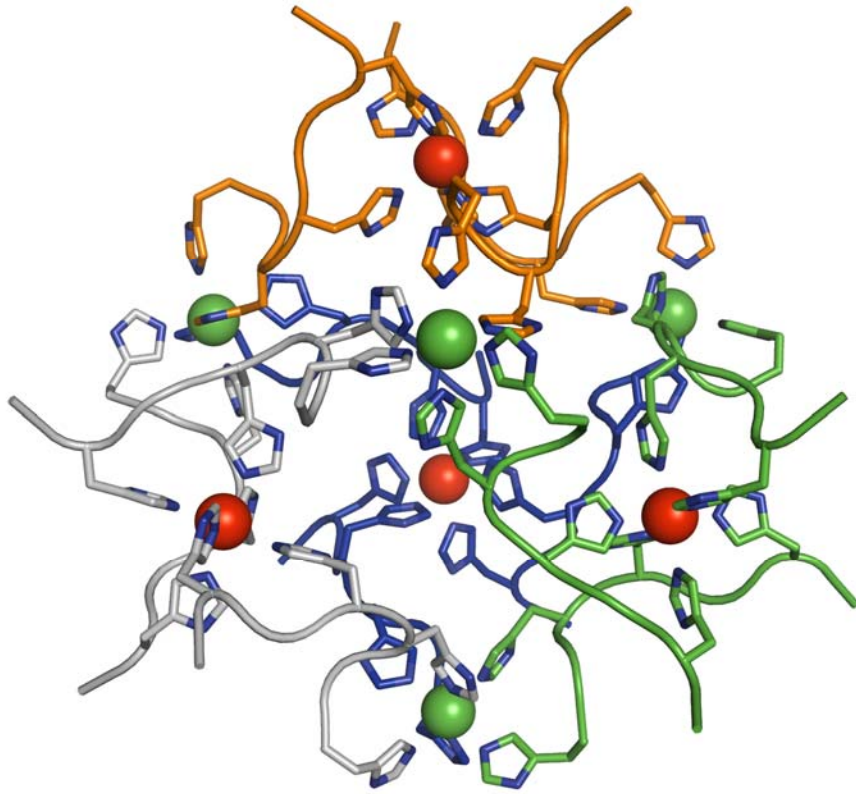


# Crystal interactions

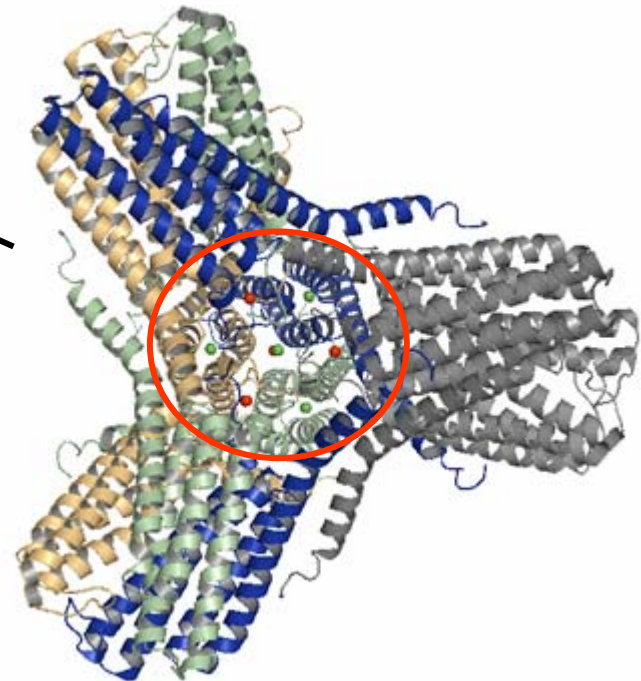
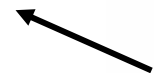
- 20% protein contents in the crystal
- His-tag metal cluster make key interaction
- Additional crystal contacts by the C-terminal helix and the cytosolic hydrophilic surface



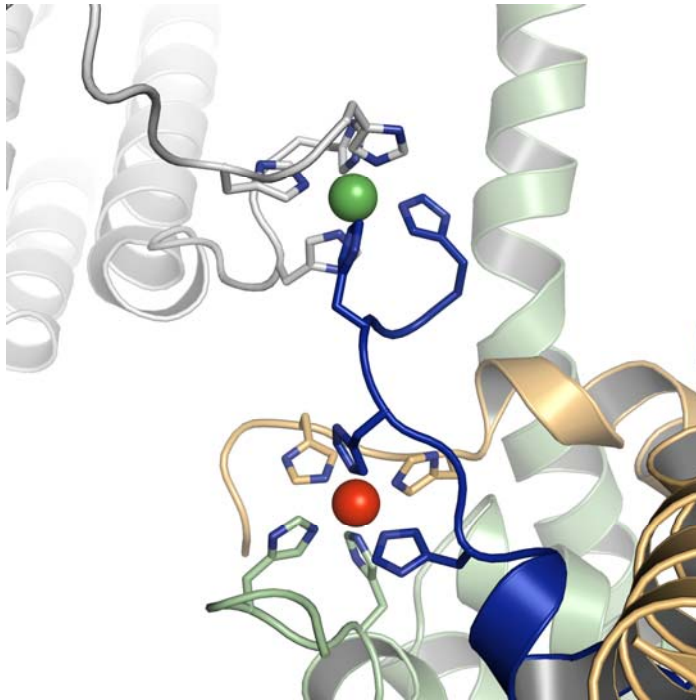
# LTC4S His-tag cluster



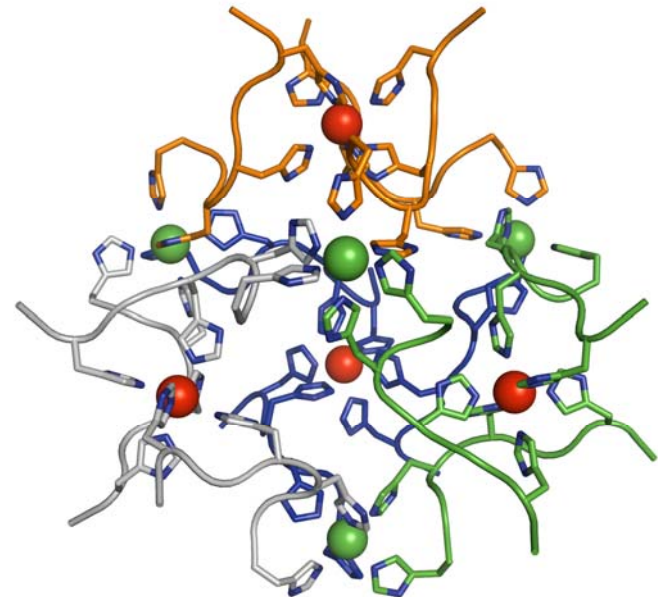
- 322 symmetry
- 12 times 6xHis tags
- 4 out of 6 His coordinate
- 8 metals (presumably Ni)



# cont. LTC4S His-tag cluster



- Each His-tag form:
- one “intra trimer center” (red)
  - one “inter trimer center” (green)



# A (relative beginners) perspective on strategies for IMP structural biology

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- Feasibility for recombinant IMP production is OK, but strategies to generate more variants still needed
- Efficient and generic (small-scale) method for protein characterization and stabilisation need.
- Extended strategies for protein surface (crystal contact) engineering need
- Availability of appropriate synchrotron stations of outstanding importance
- A component of the slow progress on IMPs might be the “Himalaya factor” – leading to less funding in the field

# Acknowledgements

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- Damian Niegowski
- Tobias Cornvik
- Pär Nordlund

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- Andrew McCarthy

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BESSY, Berlin (ID14)

SLS, Villingen, (X06SA)

MaxLab, Lund (ID7-11)

# The Membrane Protein Team



**Marie Hedren**



**Said Eshaghi**



**Daniel M. Molina**

Mol Biol => Proteins => Structure



**Marina I. Sabet**



**Andreas Kohl**



**Damian Niegowski**

**Also; Victoria Liu  
Tobias Cornvik**

Special thanks to: Scott Lesley GNF/JCSG, San Diego:

Thank you for your attention !



**Postdoc positions available:**

**Contact Pär Nordlund ([Par.Nordlund@mbb.ki.se](mailto:Par.Nordlund@mbb.ki.se))**